

# Electrophysiological Indication of Stability of Woody Plants to Industrial Pollutants: Part A

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## Abstract

Electrophysiological express method of bioelectrical potential (BEP) recording was presented to indicate stability of coniferous and deciduous trees against vapors of styrene, formaldehyde and acetone within concentration range 2-17 MPC, which are components of industrial emissions of furniture enterprises. Assessment on stability was determined by dynamics of BEP changes during daylight hours in comparison to control samples. A statistical analysis on obtained results of BEP measurements was done and universal dependence of BEP-values on time was obtained. Level of stability of metabolic systems was assessed by values of bioelectric reaction (BER). More significant impact of fumigants on seedlings grown in an open ground has been observed, compared with seedlings under laboratory conditions. Seedlings were more sensitive to pollutants than shoots. Based on the BER-coefficients it was found that maximal inhibition suffer of the test plants from exposure to toxic fumigants formaldehyde and styrene. Acetone has no adverse effect on the stability of trees.

## Keywords

*Biopotential; Coniferous and Deciduous Plants; Seedlings and Green Cuttings; Fumigation; Pollutants*

## Introduction

Electrophysiological methods have been successfully used to assess the resistance of plants to external irritants (Kolovsky, 1971). At the beginning of the 20<sup>th</sup> century, it was found that materials with a high level of metabolism were more electronegative than inert ones.

The physiological state of a tree can be determined by using the bioelectric potential (BEP), which is a quantitative measure of the qualitative changes taking place in the tree and determines further activity of the tree (Kolovsky, 1980).

It is known that potential difference arises, firstly, due to oxidation-reduction processes, and secondly, due to an uneven distribution of ions. The causes of ions asymmetry are ionic potentials, divided into diffusion,

interfacial and membrane potentials. Currently, membrane theory is leading and well-established theory, based on the totality of micro electrophysical studies. According to the theory, the underlying cause of ions asymmetry in biological systems is the presence of semi-permeable membranes limiting mobility of specific ions. The value of membrane potential difference of one type ionic flow is approximately described by the Nernst equation. Note, however, that the most significant contribution to the overall flow of ions, which determines the membrane potential, makes not a single ion, but several types of them, mostly K<sup>+</sup>, Na<sup>+</sup>, Cl<sup>-</sup>. Holdman (Junge, 1981) took into account selective permeability of membrane to these ions and derived an equation expressing value of membrane potential of cells:

$$E_M = \frac{RT}{F} \ln \frac{P_K [K^+]_{int.} + P_{Na} [Na^+]_{int.} + P_{Cl} [Cl^-]_{ext.}}{P_K [K^+]_{ext.} + P_{Na} [Na^+]_{ext.} + P_{Cl} [Cl^-]_{int.}} \quad (1)$$

where  $P_K$ ,  $P_{Na}$ ,  $P_{Cl}$  – membrane permeability of K<sup>+</sup>, Na<sup>+</sup>, Cl<sup>-</sup>;  $[K^+]_{int.}$ ,  $[Cl^-]_{int.}$ ,  $[K^+]_{ext.}$ ,  $[Cl^-]_{ext.}$  – internal and external concentrations of ions of K<sup>+</sup> and Cl<sup>-</sup> respectively.

The question of chemical side, as well as energy supply mechanisms of active transport of ions across the membrane has been currently intensively studied. The theoretical basis of this research is the chemical-osmotic concept of P. Mitchell (Mitchell, 1974). According to this theory, chemical energy of respiration and photosynthesis initially become a form of electrical and osmotic, and then reverts again to chemical (ATP synthesis). Under existing classification, biopotentials and bioelectric phenomena are referred to following groups: slow and fast; local and extending; stationary and non-stationary. Additionally, such phenomena as resting potentials, potentials of damage, action and metabolic potentials have been most often described depending on the nature of electrogenesis (Medvedev, 1997).

Thus, the electrically active part of the cell that converts chemical energy into electrical energy is the

cell membrane. The processes taking place in membrane determines biomagnetic fields or biomagnetic signals (Vvedenskiy, 1986), that is, the magnetic fields generated by an electric current flowing inside the organism during its life. For potassium  $E_{K^+} = -75$  mV, for sodium  $E_{Na^+} = +55$  mV. Outside the cell, the potential is taken as zero. If the membrane potential  $E_M$  becomes the standard, passive currents of potassium and sodium in the membrane must balance each other.

In the absence of excitation  $P_{Na} < P_K$  and  $E_M \sim E_K \sim$  and  $\sim 70$  mV, i.e. the inner side of the membrane is negatively charged. By changing the membrane permeability, conductivity of open channel is about of  $10^{-10}$  Ohm $^{-1}$ ·m $^{-2}$ . Under the state of rest, potassium channels are open and provide conductivity of membrane unit area  $P_K$  of about  $10$  Ohm $^{-1}$ ·m $^{-2}$ . Conductivity is affected by transmembrane electric field of  $10^7$  V/m, which is close to the breakdown field of a good insulator. If the cell is set out of electrical balance, then  $P_K$  increases by 10 times and  $P_{Na}$  increases from 0 to  $300$  Ohm $^{-1}$ ·m $^{-2}$ . This leads to depolarization and rise of electric pulse with duration of 1 ms. But for all that potential difference changes from -70 to + 50 mV, i.e. repolarization of cell takes place, and then returns back to equilibrium. This is the so-called action potential (AP).

Apart from influence of electric field, ion channels can be poisoned by action of chemical substances. Some poisons can completely block channels; while the others have a temporary effect (Adygezalov, 1987).

The aim of this work was at the application of electrophysiological methods of biopotential determination as a test to assess effect of industrial gas emissions of furniture enterprises on stability of coniferous and deciduous trees.

## Materials and Methods

### Samples of Woody Plants

Object of investigation included the most common wood species grown in Central Black Earth region of Russia: conifers-Scotch pine, *Pinus silvestris*, Spruce fir, *Picea excelsa*, European larch, *Larix decidua*; and hardwoods - English oak, *Quercus robur*, Silver birch, *Betula Pendula* Roth.

Green cuttings coeval plants, and one and two-year-old seedlings of trees were used for the study. Cuttings of medium size in length (one-two buds) and thickness were uniformly selected from experimental trees in an

amount of 10 grafts from the tree and placed in water.

Scots pine seedlings were grown in an open ground in the breeding nursery of the Central Research Institute of Forest Genetics and Plant Breeding (Voronezh, Russia). 80 pine seedlings selected for the experiment were divided into four groups (by 20 seedlings in each). Two-year old seedlings of English oak were planted in laboratory conditions. 10-20 seedlings of oak were selected for the experiment.

### Fumigation of Woody Plants in the Atmosphere of Industrial Toxicants

Assessment on plant resistance to the influence of industrial pollutants was carried out in fumigation chamber under laboratory conditions and in open field in arboretum.

For fumigation of woody plants samples, a fumigation chamber made out of glass with a volume of  $0.5$  m $^3$  was used, and saturated with vapors of one of industrial toxicants that were found in industrial emissions of two furniture enterprises in Voronezh (Russia): acetone (Maximal permissible concentration of maximal single dose (MPC $_{m.s.d.}$ ), MPC $_{m.s.d.}$ = $0.35$ mg/m $^3$ ), styrene (MPC $_{m.s.d.}$ = $0.003$  mg/m $^3$ ) or formaldehyde (MPC $_{m.s.d.}$ = $0.035$  mg/m $^3$ ).

The range of pollutants concentrations in chamber was determined by calculating the surface concentration of pollutants at 10, 50, 100, 500 and 1000 m from furniture enterprises in Voronezh, Russia. The maximum concentration of these pollutants was achieved at a distance of 100 m from the emission source and varied from 2 to 17 MPC $_{m.s.d.}$ . A similar concentration of toxicant was kept in the fumigation chamber equipped so that it allowed fixing the temperature (22-26°C), humidity (50%) and light (6 lux). A group of investigated plants was placed in a chamber for fumigation during 4-5 hours.

Cuttings and seedlings of conifers were fumigated in vapors of acetone and styrene. Investigated cuttings, with length 15-20 cm, were placed in 100 ml of distilled water and then placed in fumigation chamber in the atmosphere of specified toxicant. Control samples were held under favorable conditions ( $W=80\%$ ,  $t=22^\circ\text{C}$ ) for 24 hours. After that, 5 mg of KCl were added to water, contacting with cuttings, and BEP were measured.

Exposure to formaldehyde gas has also been studied on two-and four-year-old pine seedlings. Samples of hardwoods (oak and birch) were exposed to styrene vapor. For fumigation of seedlings in arboretum,

fumigation chamber, made out of wooden frame and covered with polyethylene, was placed around the testing plants.

### Measurement of BEP

BEP measurements were carried out in normal conditions, during fumigation and after a certain time after fumigation.

Registration of BEP of plants is based on measurement of a rest potential of plants as an interphase potential in system "soil-plants" (Medvedev, 1997; Vvedensky, 1986). Due to this, recording of the BEP was performed by "cambium-to-earth" scheme which has significant advantage in comparison to the scheme "cambium-cambium" because it allows comparing bioelectrical activity of individual trees in relation to total electrical standard - "the earth", i.e. evaluating them on this indicator.

For measurement of BEP universal voltmeter V7-21A with input impedance  $2.5 \cdot 10^{11}$  Ohm was used. This met following necessary requirements for:

1. Having high input impedance, which is many times higher than resistance of living tissue.
2. Absence of tissue polarization caused by registration unit.
3. Amplification of electromotive force (EMF) of tissue for reliability of reading data.

Two silver chloride electrodes, Ag/AgCl, KCl, of EVL-1M-type were chosen so that their own EMF difference was not more than 2 mV. Solution of potassium chloride was chosen due to close mobility of  $K^+$  and  $Cl^-$  ions (Kolovsky, 1980) ( $K^+ = 7,64 \frac{\mu m/s}{V/sm}$ ,  $Cl^- = 7,91 \frac{\mu m/s}{V/sm}$ ),

which prevents occurrence of diffusion potential at the interface of electrolytes. First measuring electrode was applied to leave of a plant, the second silver chloride electrode contacted through the wet porous material to the ground.

The use of high-resistance registering units allows recording of potential for green cuttings or biennial seedlings by contact manner without damage. In this case, the indifferent electrode is grounded. The most successful recording of BEP can be realized during intensive growth of needles, leaves or shoots, when tissue of young, softwood is characterized by the lowest electrical resistance. Upon the aging, resistance of plant organs and tissues increases and registration of potential without damage becomes impossible. Therefore, the most favorable period for recording of BEP is vegetation time, from May till June. Reading of

potential has been carried out from the top of cuttings or seedlings (point of growth or adjacent area).

To improve electrical contact of plant tissue with measuring electrode and to protect the plant (which causes development of action potential), a foam-rubber clamp, impregnated in 0.1 N KCl, was applied to the area of registration. Operation with conifer cuttings or seedlings is complicated by fully developed needles that impede clamps pressing. Due to this, the needles were stripped on a limited area, resulting in additional irritation of plant and development of action potential. In this case, the plants were included in experiment no less than 24 hours later, keeping them to restore normal physiological activity under more comfortable conditions ( $t = 18-20^\circ C$ , 60 - 70% of air humidity, indirect lighting). In other cases, after pressing the clips, the plants are ready to work after 1-2 hours.

Assessment on plant resistance to gaseous pollutants was made by dynamics of BEP measurement during daylight hours, in relation to control samples.

Within each type of plant cuttings or seedlings bioelectric response (BER) was calculated (Shevernozhuk, 1989). The BER reflects resistance level of metabolic systems of plant against the impact of stressful irritant, i.e. high gas-pollution of the medium. Calculation of the BER was done using the equation:

$$BER = \pm \frac{(\varphi - \varphi_c)}{|\varphi_c|}, \quad (2)$$

where  $\varphi$  - biopotential of fumigated sample in the measurement phase, mV;  $\varphi_c$  - biopotentials of control samples, mV.

The measurements of BEP were carried out during daylight hours, because in this time period (9-15 hours) the rate of photosynthesis changes significantly and gas resistance is minimal.

In the absence of fumigation, dynamics of BEP changes for cuttings and seedlings during daylight characterizes normal operation of metabolic systems of plants. It is to register a potential shift to negative values in the period from 9 to 12 am, the maximum negative value of the potential in the next 12 hours and the potential shift are towards more positive values.

Effect of plants fumigation by toxicants has been tested in an abnormal reaction of plants to the light, determined from changes of BET from the control. Increasing values of BER shows a significant effect of fumigants on physiological processes. The negative sign reflects that the BER stimulates metabolic processes, positive-reduction of these processes in

comparison with the control. BER tends to zero, if there is almost no effect of fumigants on stability of trees.

## Results and Discussions

### *Bioelectrical Reaction of Conifer Cuttings by Fumigation in Acetone and Styrene*

Resistance of cuttings of Spruce fir, Scots pine and European larch to acetone and styrene was assessed. Groups of 30 cuttings of each plant species were included in experiment. Resistance of tree species under study to toxicants was determined from change of BEP of plants placed in the fumigation chamber with closed fumigation cycle, which allowed fumigating plants in a stream of air contaminated with styrene and acetone. Fumigation of experimental cuttings was carried out at a concentration equal to 17 MPC<sub>m.s.d.</sub>

It was observed that in atmosphere of acetone, activation of physiological processes takes place and remains one day after fumigation. Treatment of spruce cuttings with styrene caused the phase of anxiety in plant; BEP increased and then abruptly shifted in a positive direction during daylight hours, which indicates depression of biological functions of plants.

On the first day after fumigation of spruce cuttings in styrene BEP smooth transitioned to more negative values (from 9 to 15 hours), i.e. transition of plant from the phase of depression into phase of stimulation was realized. Abnormal response of plant to acetone remained during daylight hours in a day after fumigation.

Effect of styrene is opposite to that of acetone. Styrene causes depressing effect and restoration of vital processes does not occur neither on the day of fumigation, nor on the day after fumigation.

TABLE 1 RELATIVE COEFFICIENTS OF BIOELECTRIC REACTION (BER) OF SPRUCE, PINE AND LARCH CUTTINGS

Conditions	Wood species					
	Spruce		Pine		Larch	
	$\frac{A}{A^1}$	$\frac{S}{S^1}$	$\frac{A}{A^1}$	$\frac{S}{S^1}$	$\frac{A}{A^1}$	$\frac{S}{S^1}$
t, °C	$\frac{23}{26}$	$\frac{23}{26}$	$\frac{23}{26}$	$\frac{23}{26}$	$\frac{23}{26}$	$\frac{23}{26}$
Humidity, %	$\frac{58}{56}$	$\frac{58}{56}$	$\frac{58}{56}$	$\frac{58}{54}$	$\frac{58}{56}$	$\frac{58}{56}$
BER	$\frac{-0.35}{-0.18}$	$\frac{+0.18}{+0.06}$	$\frac{-0.075}{-0.28}$	$\frac{+0.28}{+0.21}$	$\frac{-0.17}{-0.53}$	$\frac{+0.38}{+0.22}$

Note: A – fumigation in acetone; S – fumigation in styrene; A<sup>1</sup> и S<sup>1</sup> – measurement of BEP after 24 h from fumigation in acetone (A<sup>1</sup>) and styrene (S<sup>1</sup>). Time of BEP measurement 12:00 a.m.

Thus, this study confirmed different reactions of pine, spruce and larch on exposure of acetone and styrene. Quantitative expressions of softwood response to fumigants are presented in table 1 as relative ratios of BER.

Analysis of BER values showed that acetone stimulated metabolic processes in cuttings of spruce, pine and larch on the day of fumigation, and this effect persisted for the next day (BER takes negative values). To a large extent activating effect of acetone was reflected on the cuttings of spruce, then larch and pine. However, on the next day of effect of acetone increased to a greater extent on larch: BER value increased from -0.17 to -0.53. Also, there was an increase in BER for pine from -0.075 to -0.28, but for spruce BER reduced from -0.35 to -0.18 (Table 1).

Styrene causes depressing effect for all wood species. On the next day after fumigation, there is no restoration of vital functions of cuttings (table 1). Spruce was depressed to a lesser extent, then pine. Larch expressed the strongest stress from exposure to styrene (BER = + 0.38).

### *Bioelectrical Reaction of Annual Seedlings of Spruce, Pine and Larch on Processing In Vapors of Acetone and Styrene*

As the next step, bioelectric reactions of annual seedlings of spruce, pine and larch on processing in vapors of acetone and styrene by the same concentration of 17 MPC of pollutant were studied. Study of dynamics of annual seedlings of these species of conifers was carried out in fumigation chamber under normal conditions. It was shown that the spruce, pine and larch revealed normal reaction to the light during daylight hours that confirms normal operation of metabolic systems of studied plants.

TABLE 2 RELATIVE COEFFICIENTS OF BIOELECTRIC REACTION OF ANNUAL SEEDLINGS OF SPRUCE, PINE AND LARCH

Wood species					
Spruce		Pine		Larch	
$\frac{A}{A^1}$	S	$\frac{A}{A^1}$	S	$\frac{A}{A^1}$	S
$\frac{+0.073}{-0.048}$	+0,22	$\frac{+0.11}{+0.066}$	+ 0,33	$\frac{+0.12}{+0.14}$	+0,50

Note: A – fumigation in acetone; S – fumigation in styrene; A<sup>1</sup> и S<sup>1</sup> – measurement of BEP after 24 h from fumigation in acetone (A<sup>1</sup>) and styrene (S<sup>1</sup>). Time of BEP measurement 12:00 a.m.

Studying BEP dynamics for seedlings of spruce, pine and larch in the atmosphere of acetone and on the next day after fumigation showed that seedlings of conifer species were inhibited by vapors of acetone to a small extent, and some restoration of normal operation was

observed only for spruce seedlings (table 2).

Styrene acts as a stress factor and plant inhibition increases in a row spruce → pine → larch (Table 2).

Seedlings of spruce, pine and larch revealed stronger response signal to exposure of acetone and styrene than cuttings of these species (Table 1 and 2).

For shoots and seedlings, transition to different phases of stress reaction of plant has occurred. By fumigation of conifers shoots in acetone, it is traced phase of anxiety and stimulation phase; by processing in styrene vapors, phase of depression was observed. For seedlings, only the third phase-the depression was observed, but its extent depended on the nature of fumigant and species of seedlings. The degree of inhibition by exposure of styrene was much greater and to greater extent it appeared on larch seedlings (Table 2).

The stability of different conifer crops to organic gaseous toxicants was determined in (Belchinskaya et al., 1991, 1994<sup>a</sup>; Mezentseva et al., 1990; Krasnoboyarova et al., 1991). It was proved that there cannot be plants completely stable or unstable to industrial emissions. Plants adapt to the pollutants, first of all, due to diverse adaptation mechanisms formed in plants during phytogenic development. Moreover, the more coping mechanisms are used by the plant at the same time on different levels, the more resistant organism is to the action of individual ingredients of pollution and their components.

#### ***Stability of Biannual Pine Seedlings by Processing With Acetone, Styrene and Formaldehyde***

Before starting the fumigation, dynamics of BEP biennial pine seedlings grown under normal conditions, were studied. The mean values of BEP in the four groups of experimental plants had approximately the same values 55.4-56.7 mV, which indicated the same level of biological activity of seedlings selected for fumigation. In further studies, the first group served as a control sample, the second group of pine seedlings was placed in an atmosphere saturated with vapors of acetone; the third one-in the atmosphere with high concentrations of styrene; and the fourth-in formaldehyde.

Fumigation of seedlings was carried out at concentrations of acetone, styrene and formaldehyde corresponding to 2 and 17 MPC<sub>m.s.d.</sub> for each pollutant. General observations of the state of plants were carried out for two months after fumigation. In normal conditions, investigated plants (control samples) had

normal responses to the light during daylight hours.

By fumigating in styrene, acetone and formaldehyde, the most pronounced anomalous behavior of seedlings was found in case of formaldehyde. Only on the second day after fumigation, their behavior was close to the control one, which is probably related to protective functions of plants caused by stress condition after fumigation. It was shown in (Belchinskaya et al., 1990) that formaldehyde caused inhibition of photosynthesis in plants during the daylight hours. The possibility of three-phase changes in intensity of photosynthesis and two-phase breathing by the fumigation was also suggested in (Belchinskaya et al., 1990).

Changes of BEP for pine seedlings during fumigation in styrene included their anomalous behavior on the day of processing in styrene vapors, while on the next day the behavior of seedlings became more similar to controls. Such a reaction of plant to the impact of styrene showed low resistance to pine this toxicant. Similar negative reaction to contamination of atmosphere by styrene was observed in studies with shoots and annual seedlings of pine in laboratory conditions.

Processing of seedlings with styrene at concentration of 2 MPC<sub>m.s.d.</sub> made behavior of plant change not as greatly as during fumigation at styrene concentration of 17 MPC<sub>m.s.d.</sub> On the first day after fumigation in styrene vapors at 2 MPC<sub>m.s.d.</sub>, the behavior of seedlings is similar to that for control seedlings.

The least impact on biological activity of Scots pine seedlings caused acetone, which corresponded to its higher MPC<sub>m.s.d.</sub>=0.35 mg/m<sup>3</sup> and the hazard class IV. Although some shift of BEP to positive values during fumigation was marked, the variation of the potential on time was almost not affected.

TABLE 3 RELATIVE COEFFICIENTS OF BIOELECTRIC REACTION OF BIENNIAL PINE SEEDLINGS

Conditions	Fumigant			
	Acetone	Styrene		Formaldehyde
	17 MPC	2 MPC	17MPC	17 MPC
Fumigation	+ 0,25	+ 0,36	+ 0,50	+ 0,38
On the first day after fumigation	+ 0,10	+ 0,31	+ 0,47	+ 0,29
On the second day after fumigation	-	-	-	- 0,066

Note. Measurement of BEP at 12:00 a.m.

Thus, different bioelectrical reaction of biennial pine seedlings grown in an open air in arboretum on gas

pollution of atmosphere was determined by volatile organic compounds: acetone, styrene and formaldehyde.

According to values of BER-coefficients, pine seedlings suffered the greatest inhibition from exposure of styrene and formaldehyde which are the most toxic air pollutants in industrial emissions of furniture factories. Least of all biennial pine seedlings responded on pollution of atmosphere by acetone and quickly restored their vital functions after fumigation.

Based on the values of BER, inhibition of plant is quantified by about 50% of control by exposure of styrene (BER = +0.50) and slightly lower during fumigation with formaldehyde (BER = + 0.38). Treatment of seedlings with acetone also showed the element of stress, but not so strong (BER = + 0.25).

The observations of seedlings from this series of experiments, as reported above, were continued without metering the BEP within the next two months after fumigation. During this time, all of the plants developed normally: however, drying or depression was not observed.

#### **Identification of State of 2-Year-Old Seedlings and Sprouts of Oak and Birch by Processing with Styrene Vapors**

Hardwood trees occupy large areas in Voronezh and adjacent areas (Russia). Oak forests in the Central Black Earth region of Russia occupy more than half of the area covered by the main tree species, and in some areas their area reaches 75-78%. Oak forests in this region are one of the most important components of the biosphere and widely used for various purposes. The area occupied by birch forms 10% of forest fund available in the area. However, growing in forest-poor and densely populated area of the European part of Russia, oak and birch are exposed to human impacts related to human activity in industry, which negatively

affects their growth and condition (Kulagin, 1974; Getko, 1989; Artamonov, 1986). Therefore, the task of this study was directed to testing of electrophysiological method for physiological monitoring of forest under exposure to styrene and acetone vapors, contained in gas emissions, for seedlings and cuttings of green English oak, *Quercus robur*, and Silver birch, *Betula Pendula Roth*, located in zone of intoxication. Short-term stress conditions were simulated by exposure of toxicant under dose of 17 MPC<sub>m.s.d.</sub>

Laboratory studies of two-year-old oak seedlings showed that both toxicants cause inhibition of vital activity functions of plants, but the effect of styrene was stronger, indicating anomalous change of  $\phi - \tau$  dependence. However, after 6 days and fumigation with acetone and styrene, there was not only a full recovery of fumigation activity of oak, but there was some activation of it in comparison with control.

As a result of laboratory study of functional activity of oak and birch shoots to styrene, it was marked deviation from a normal course of dynamic curve only on the day of fumigation. The response of shoots was significantly less than that for seedlings.

Restoration of physiological processes was observed on the following day.

Thus, our studies have shown the possibility of rapid and reliable identification of the state of two-year-old oak seedlings and sprouts of oak and birch, but the time of this process was different: the longest time was for oak seedlings by processing in vapors of styrene (Belchinskaya<sup>b</sup> et al., 1994).

Investigations on the arboretum revealed lesser decrease of functional activity of oak and birch shoots under exposure of styrene vapors in comparison with their fumigation under the same toxicant in laboratory conditions. Physiological processes fully recovered on the first day after the action of styrene vapors.

TABLE 4 RELATIVE COEFFICIENTS OF BIOELECTRIC REACTION OF TWO-YEAR-OLD SEEDLINGS AND SHOOTS OF ENGLISH OAK AND SILVER BIRCH

Conditions	Wood Species				
	English Oak			Silver Birch	
	seedlings	shoots (in lab)	shoots (arboretum)	shoots (in lab)	shoots (arboretum)
<b>Fumigation in acetone</b>					
On the first day after fumigation	+ 0,10	-	-	-	-
On the sixth day after fumigation	+ 0,22	-	-	-	-
	- 0,29	-	-	-	-
<b>Fumigation in styrene</b>					
On the first day after fumigation	+ 0,45	+ 0,28	+ 0,18	+ 0,22	+ 0,21
On the sixth day after fumigation	+ 0,44	+ 0,11	+ 0,08	+ 0,16	+ 0,03
	- 0,72	-	-	-	-

Note: BEP measured at 12:00 am.

Quantitative parameters (BER) of the impact of air pollutants, i.e. acetone and styrene, on physiological condition of seedlings and cuttings of English oak and Silver birch are given in Table 4. For calculations of BER the day time of 12:00 am were selected.

Algebraic value of BER is higher for oak seedlings (BER = 0.45). Shoots of oak and birch have more stress in under laboratory conditions than that in the arboretum (Table 4).

On the next day after fumigation in styrene, bioelectrical response of oak and birch trees in arboretum tends to zero. Thus, it was found dependences of pollutants nature and wood species on metabolism processes, and the change of which was assessed by electrophysiological method of bioelectric potentials measurement. The stress state of shoots and seedlings was determined, developing more significantly in case of processing with styrene rather than acetone. It was shown that the oak experienced slightly higher stress of physiological processes under the influence of styrene than the birch.

#### **Statistical Analysis of BEP of 2-and 4-year-old Seedlings of Scotch Pine, Treated with Formaldehyde, Styrene and Acetone**

The highest changes of BEP were observed for seedlings fumigated with formaldehyde, then styrene. Treatment with acetone practically did not reflect on the value of BEP: there was a potential shift in positive direction, which characterized the largest braking of metabolic processes. Effect of styrene and formaldehyde stress condition of the plant was observed (Fig. 1).

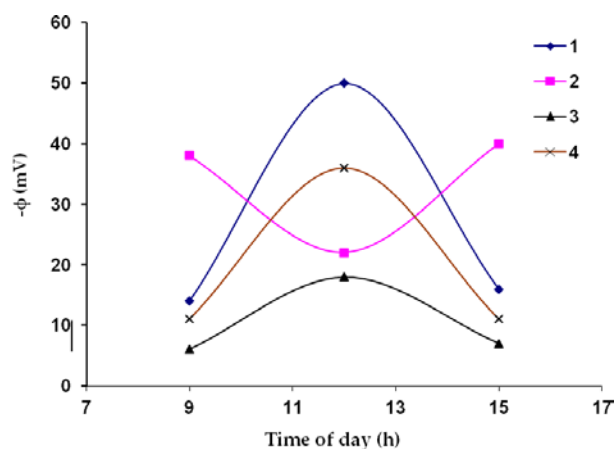


FIG. 1 DEPENDENCE OF BEP ON TIME DURING THE DAY IN ABSENCE (1) AND BY FUMIGATION OF PINE SEEDLINGS IN FORMALDEHYDE (2), STYRENE (3) AND ACETONE (4)

An attempt to obtain a universal dependence of BEP-

value on time during daylight hours was made. For this, values of BEP were normalized by BEP measured at 12:00, and the time was normalized by 12 hours, i.e. normalized biopotential was calculated

$$\varphi = \frac{\bar{\varphi}}{\varphi_{12}}, \quad (3)$$

where  $\varphi$ -normalized value of biopotential;  $\bar{\varphi}$  - average biopotential, mV;  $\varphi_{12}$  - average biopotential, measured at 12:00 am, mV.

On the base of experimental results, universal dependence of BEP-values on time during the daylight was obtained and approximated by parabolic dependence

$$\varphi(\tau) = b_0 + b_1\tau + b_2\tau^2, \quad (4)$$

where  $\varphi$  - normalized value of biopotential;  $\tau$  - normalized time;  $b_0$ ,  $b_1$  and  $b_2$  – coefficients.

Coefficients  $b_0$ ,  $b_1$  и  $b_2$  for controls and samples fumigated by formaldehyde, styrene and acetone are given in table 5.

TABLE 5 MATHEMATICAL MODELS OF PROCESSING OF SCOTCH PINE SEEDLINGS FUMIGATED BY FORMALDEHYDE, STYRENE AND ACETONE

Nature of fumigant	Universal dependence of BEP-values ( $\varphi$ ) of seedlings on time ( $\tau$ )
Control measurement*	$\varphi = -5.91 + 13.84\tau - 6.947\tau^2$
Formaldehyde -on the day of fumigation	$\varphi(\tau) = 4.80 - 7.67\tau + 3.55\tau^2$
-on the next day after fumigation	$\varphi(\tau) = -14.58 + 29.17\tau - 13.74\tau^2$
-on the second day after fumigation	$\varphi(\tau) = -3.15 + 8.6\tau - 4.45\tau^2$
Styrene -on the day of fumigation	$\varphi(\tau) = -4.59 + 9.24\tau - 4.19\tau^2$
-on the second day after fumigation	$\varphi(\tau) = -1.64 + 4.27\tau - 2.09\tau^2$
Acetone -on the day of fumigation	$\varphi(\tau) = -2.32 + 5.82\tau - 2.88\tau^2$
-on the next day after fumigation	$\varphi(\tau) = -17.19 + 33.24\tau - 19.98\tau^2$

Note: \* Mean-square error  $\sigma = 0.07$  mV.

Based on analysis of the obtained dependences, it was found that on the day of fumigation with formaldehyde coefficients  $b_0$ ,  $b_1$  and  $b_2$  have characteristics opposite to corresponding coefficients in the control group of seedlings. One day later, the characteristics of  $b_0$ ,  $b_1$  and  $b_2$  coefficients were restored, but coefficients were higher compared to the first ones, characterizing an uncertainty of this dependence and small changes in the value of potential. Two days after fumigation rates took values

similar to control group of seedlings.

On the day of fumigation in acetone coefficients  $b_0$ ,  $b_1$  and  $b_2$  corresponded to the control ones, but after a day they increased sharply in line with the above case. Finally, by fumigation in styrene coefficients  $b_0$ ,  $b_1$  and  $b_2$  were similar to corresponding coefficients of  $\phi(\tau)$ -dependence for control sample.

By discussing the effect of pollutants on bioelectric response of coniferous plants, it is necessary to consider participation of toxic substances in chemical reactions responsible for processes of photosynthesis. If a plant cell does not undergo significant changes and a toxicant may enter into it freely, there is a high probability (Belchinskaya et al., 1990; Grushko, 1986) of that acetone and formaldehyde participated in cycles of transformations into amino acids and styrene into aromatic amino acids. As a result, plants were not in a depression state for much time and almost left it to the first or second day, because during that time they overcome stress arising during fumigation in toxicants, in which concentration exceeded  $MPC_{m.s.d.}$ . During this time toxicants were included in metabolism by aerobic oxidation and plants showed higher activity of physiological processes.

Pine seedlings are indicators of short-term exposure to air pollution by styrene and formaldehyde in concentration ranging from 2 to 17  $MPC_{m.s.d.}$

In the given concentrations, acetone did not have adverse effects on Scots pine and can not be attributed to polluting organic components of atmosphere for pine seedlings.

According to values of BEP, styrene and formaldehyde cause temporary depressing effect on pine seedlings, but not overwhelming one in view of their possible involvement in chemical reactions responsible for the process of photosynthesis.

It was found that negative effects of fumigants were much more significant for seedlings than that for the shoots of Scots pine (Tables 1, 2, 3). Scotch pine seedlings grown in the open ground are more sensitive to air pollutants than the laboratory seedlings.

Thus, it is necessary to conclude that both a parabolic dependence  $\phi(\tau)$  and an algebraic value of BER should be used for discussion of the influence of toxicants on stability of trees. Without complementarities of these two characteristics, it is difficult to make correct conclusions about interaction of atmospheric pollutants on physiological condition of the plants.

## Conclusions

Thus, based on investigations and analysis of obtained results, it was found that the impact of toxic gaseous substances on physiological condition of woody plants was determined by the express method of bioelectrical potential recording that allows recognizing a bioindicative role of certain species for industrial gas emissions, including such organic compounds, such as formaldehyde, styrene, and acetone.

Thus, the complex of obtained data allows us to recommend the use of electrophysiological methods of biopotential recording for determination of the state of tree plantations with the aim of assuming the measures to prevent further adverse effects on environmental pollution.

In the next report, the focus is on the analysis of results of application of electrophysiological method of BEP measurement for assessment on stability of real systems including forestlands located nearby industrial enterprises.

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